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ISOLATION, CHARACTERIZATION, AND ANTIBIOGRAM OF DIARRHOEAGENIC BACTERIA FROM PAEDIATRIC PATIENTS IN SELECTED HEALTHCARE FACILITIES OF ENUGU STATE NIGERIA

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ABSTRACT

Diarrhoea in children is a serious condition and if not carefully handled, may lead to fatalities. In this work, we isolated, characterized, and determined the antibiotic sensitivity pattern of *E. coli*, *Shigella* spp, *and Salmonella* spp recovered from faecal samples from children's wards in selected clinics in the Enugu State of Nigeria. All the samples were transported to the laboratory using ce Park. The isolates were further sub-cultured onto Salmonella-Shigella Agar and eosin-methylene Blue Agar to obtain pure and viable colonies and stored on a Nutrient agar slant until further analysis. Each isolate was Gram-stained, and various biochemical tests were conducted. The isolates were subjected to antibiotic susceptibility tests using the disc diffusion method. The antibiotic disc used include ceftriaxone 30 µg, gentamicin 10µg, ciprofloxacin 5 µg, erythromycin 5µg, ertapenem 10 µg, vancomycin 30 µg, cefoxitin 30 µg, amoxicillin/clavulanic acid 30 µg and Tetracycline 30µg. Ninety-eight (98) *shigella* isolates were recovered from the 468 patients admitted during the study, giving a prevalence of 20.9%; one hundred and eighty-three (183) 39.1% *Salmonella* spp and one hundred and eighty seven (187) 40.0% for *E. coli* spp. The antibiotics sensitivity testing showed that the isolates tested were resistant to most antibiotics except ciprofloxacin and gentamicin. Ciprofloxacin and gentamicin produced excellent antibacterial activity against most test organisms. The result of this study underscores the importance of antibiotic sensitivity testing as a guide to precise antimicrobial therapy.

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INTRODUCTION

Diarrhoeal illnesses remain one of the most important causes of global childhood mortality and morbidity despite much progress in the understanding of its pathogenesis and management. The leading cause of morbidity and mortality in children worldwide remains infectious diarrhoea [1]. Diarrhoea results from intestinal tract infection by a wide range of enteric pathogens that can disrupt intestinal function. The global burden of infectious diarrhoea annually is enormous, involving 3 to 5 billion cases and nearly 2 million deaths, with the latter accounting for about 20% of all deaths in children younger than 5 years [2]. Of these diarrhoea-related deaths, acute watery diarrhoea is responsible for 35%, dysentery 20%, and

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persistent or chronic diarrhoea 45% [3]. Most deaths are found in young children from rural regions of developing countries where there is limited access to safe drinking water, sewage disposal, health care, and reduced opportunities for personal sanitation, hygiene, and safe food preparation [4].

The incidence of diarrhoea varies with the season and the child's age. This incidence is highest in the first two years and declines after that. In Nigeria, diarrhoea prevalence rate is 18.8% and is one of the worst in sub-Sahara Africa and above the average of 16%. Annually, it accounts for over 16% of child deaths and an estimated 150,000 deaths mainly among children under five years [5]. Each year, Nigeria records at least 151,700 diarrhoea-related child deaths attributed to poor hygiene practices, poor standard of living, high poverty and illiteracy levels, and ingestion of contaminated water and foods because of extreme hunger [6].

The agents that cause diarrhoea are usually transmitted through the ingestion of faecal-contaminated water or food, person-to-person contact, and direct contact with infected faeces [7]. Cases of diarrhoea have long-term complications like malnutrition, growth retardation, and immune impairment [8]. According to research, to effectively prevent diarrhoea-associated illness and death, evaluating and identifying the local risk factors associated with diarrhoea should be the first line of action in local communities [8].

Diarrhoeagenic *Escherichia coli* has been identified as the common bacterial pathogen causing diarrhoea disease among children [9]. *E. coli* strains that cause diarrhoea have evolved by acquiring, through horizontal gene transfer, a particular set of characteristics that have successfully persisted in the host [10]. *Shigella* spp threats to public health secondary to their implications in childhood diarrhoea are documented [11, 12]. While Salmonella spp may not be exonerated, its public health burden as a diarrhoea-causing organism is low compared to other organisms implicated in diarrhoea [13]. In this study we isolated, characterized, and carried out antibiogram studies on *E. coli*, Shigella spp, and *Salmonella* spp implicated in diarrhoea in selected hospitals in Enugu State.

MATERIALS AND METHODS

Materials

All antibiotics discs used for the study were procured from Himedia (Mumbai, India), and the antibiotics include ceftriaxone, gentamicin, ciprofloxacin, erythromycin, ertapenem, vancomycin, cefoxitin, amoxicillin/clavulanic acid, tetracycline. The media used are MacConkey agar, Salmonella Shigella agar (Titan Biotech Ltd. India), Selenite/F broth, and Simmons citrate agar were used. All the reagents used for analysis were of analytical grade. All media were prepared according to the manufacturer's instructions except otherwise stated.

Inclusion and Exclusion Criteria

Inclusion criteria: children who were clinically diagnosed as suffering from diarrhoea, children who presented with the signs and symptoms of enteric fever such as splenomegaly, diarrhoea, vomiting, abdominal pain, palpable masses, hepatomegaly, anorexia among others; patients who for at least 14 days before hospital visit had not taken any antibiotics were included in this study upon consent. Exclusion criteria: Patients having fever ≥ 37.5°C for three days and their blood slides for malaria parasites were negative. Patients with no signs or symptoms of enteric fever were excluded from this study.

Ethical approval and clearance certificates for the study were granted by the Health Research Ethics Committee of the University of Nigeria Medical Center Nsukka FPSRE/UNN/20/0023., Bishop Shanahan Hospital Nsukka IRB/HEC No S. 314 (protocol no 891), Faith Foundation Mission Hospital Nsukka FPSRE/UNN/20/0023 and Enugu State University Teaching Hospital, Paklane, Enugu {Certificate numbers: NHEC/05/01/2008B-FWA00002458-IRB00002323

Sample Collection and Characterization

Five hundred (500) samples were collected from four hospitals in Nsukka and Enugu: University of Nigeria Medical Center, Faith Foundation Mission Hospital, Bishop Shanahan Mission Hospitals, and Enugu State Teaching Hospital (Parklane). All the insulation was collected between the period of December 2022 and April 2023. The samples were transported to the laboratory within 3 h of collection for processing. Different media were used for the isolation of *E. coli, Shigella, and Salmonella spp*. The different isolates were subjected to biochemical tests. The bacteria strains were maintained on Nutrient agar slants.

Antibiogram Assay

Pure isolates of *Salmonella, E. coli,* and *Shigella spp.* were inoculated on sterile Nutrient Agar (NA) plates using the agar streak plate method and incubated at 37°C for 24 h, sterilized water was aseptically dispensed into sterile test tubes. A colony of the isolate was picked and dispersed in 5 ml of sterile water. This was compared to the 0.5 McFarland standard. The turbidity was adjusted to 5 % McFarland standard by adding not more than four colonies into each dispersion.

The stored culture of the bacteria was swabbed on the prepared Mueller Hinton agar plates using sterile swab sticks. The plates were allowed to dry, and the antibiotic discs (ceftriaxone 30µg, gentamicin 10µg, ciprofloxacin 5µg, erythromycin 5µg, ertapenem 10µg, vancomycin 30µg, cefoxitin 30µg, amoxicillin/clavulanic acid 30 µg and tetracycline 30 µg.) were placed on the surface of the swabbed Mueller-Hinton plates. The plates were left for 30 minutes to allow the antibiotic discs to properly impregnate the agar before incubating at 37°C for 24 h. The zones of inhibitions on the plates were measured and recorded.

RESULTS

The distribution of isolates from various health facilities is shown in Figure 1. The numbers of isolates are as follows:

Bishop Shanahan Hospital (161), Parklane (145), University Medical Center (125), and Faith Foundation Hospital (69). Similarly, the prevalence of the three diarrhoeagenic bacteria of interest is presented in Figure 2.

Biochemical Characteristics of *E. coli* Cultured from Pediatric Ward of Four Hospitals

The biochemical characteristics of *E. coli* cultured from pediatric wards of four hospitals are presented in Table 1. The morphological characteristics observed for each bacterial colony after 24 h of incubation include colony appearance, shape, elevation, edge, optical characteristics, consistency, colony surface, and pigmentation. It was observed that the morphological characteristics of each bacterial culture differed from the other isolates. The *E. coli* culture showed slight growth, non-mucoid, and a pink to green metallic sheen with a characteristic dark center. In addition, the *E. coli* culture tested positive for eosin methylene blue (EMB), indole, and methyl red (MR) but was uniformly negative for Voges- Proskauer (VP), citrate, and urease tests. These features are characteristics of *E. coli* organisms in the culture.

Biochemical Characteristics of *Shigella spp* isolated from Four Paediatric Ward

The results of the biochemical characteristics of *Shigella* spp isolates from four paediatric wards showed parallel negative results for motility, urea, oxidase, hydrogen sulfide, and citrate and were also Gram-negative.

Biochemical Characteristics of *Salmonella spp* Isolated from Four Pediatric Ward

The biochemical characterization of *Salmonella spp*. from children's faecal samples showed that all isolates are Gram-negative, and indole-negative, and most of the isolates are positive for citrate, triple sugar iron test, sulfur test with a few negatives for the last three tests. The isolates are all motile.

Antibiotics Susceptibility pattern of E. coli in percentage

The results of the *E. coli* antibiogram are shown in Figure 3. Results show that the *E. coli* isolates are susceptible to ertapenem, cefoxitin, gentamicin, and ciprofloxacin at various degrees. However, the isolates were resistant to vancomycin, cefotaxime, erythromycin, tetracycline, and amoxicillin/clavulanic acid. Therefore, treatment of *E. coli* infection should be affected using drugs to which the organism showed high susceptibility. The interpretation in terms of resistance, intermediate, and sensitivity was based on the CLSI, multi-antibiotic resistance index (MAR INDEX).

Percentage Distribution of Antibiotics Susceptibility of *Shigella* spp

The results showed that the organism was resistant to ertapenem, vancomycin, ceftriaxone, cefoxitin, and erythromycin but completely susceptible to ciprofloxacin and mid-way susceptible to gentamycin Figure 4.

Percentage Distribution of Antibiotics Susceptibility of *Salmonella* spp

The organism was utterly resistant to ertapenem, vancomycin, and erythromycin but completely susceptible to ciprofloxacin and mid-way susceptible to gentamycin and amoxicillin/clavulanic acid (Fig 5). The result also showed ciprofloxacin (79.63), gentamicin (66.67%), amoxicillin/clavulanic acid (34.04%), and, to a lesser extent, tetracycline (16.67%) as promising antibiotics in paediatric salmonella infections.

DISCUSSION

Diarrhoea is a severe and sometimes lethal disease condition characterized by recurrent episodes and copious discharge of intestinal content in the form of loose or watery stools [14]. The discharge happens as a response to increased stomach motility, and in return, sufficient fluid is lost because there is no replacement, it leads to dehydration, loss of electrolytes, and death [15, 16]. Babies who feed on breast milk often experience watery stools because they have not developed the enzyme system to digest milk. Such a condition does not qualify as diarrhoea [17, 18]. A condition is described as diarrhoea if the diarrhoeal organism is present and continually stimulates the gastrointestinal tract to empty the content [19, 20]. The diarrhoeal pathogens are excreted with faecal materials, making faecal materials an ideal source of diarrhoeal pathogens [21].

The high global prevalence rate of diarrhoea (2.5 billion) among under-five children per year, with about 800,000 deaths, necessitates a rapid search for effective treatment [22]. In this study carried out using representative hospitals in Nsukka and Enugu town in Enugu State, Nigeria, we narrowed it down to the burden of diarrhoea in children 0-5 years caused by three bacteria implicated in diarrhoea, namely *Escherichia* spp, *Shigella* spp, and *Salmonella* spp. Therefore, the pediatric faecal samples from these health facilities represented an excellent cocktail of the bacteria-causing diarrhoeal organisms in Nigeria's Nsukka and Enugu.

Earlier studies had shown that bacterial organisms with a known history of acute diarrhoea are those of *E. coli* origin, especially entero-pathogenic *E. coli*, entero-invasive *E. coli*, verocytotoxin-producing *E. coli*, entero-aggregative *E. coli*, entero-toxigenic *E. coli*, and attaching and effacing *E. coli* [23, 24]. Other diarrhoealgenic bacterial species include *Salmonella* and *Shigella* species. *Salmonella* species include *S. Typhimurium, S. enterica, S. infantis, S anatum, S. Newport*, and S. *Ohio* (25). Meanwhile, *Shigella* species include *Shigella flexneri, Shigella sonnei, and S. boydii*. These three diarrhoeal-causing pathogens were identified in this study, suggesting their possible mediation in child diarrhoeal cases [26].

Very importantly, more than 90% of all reported cases of diarrhoeal infections are caused by bacteria; other organisms,

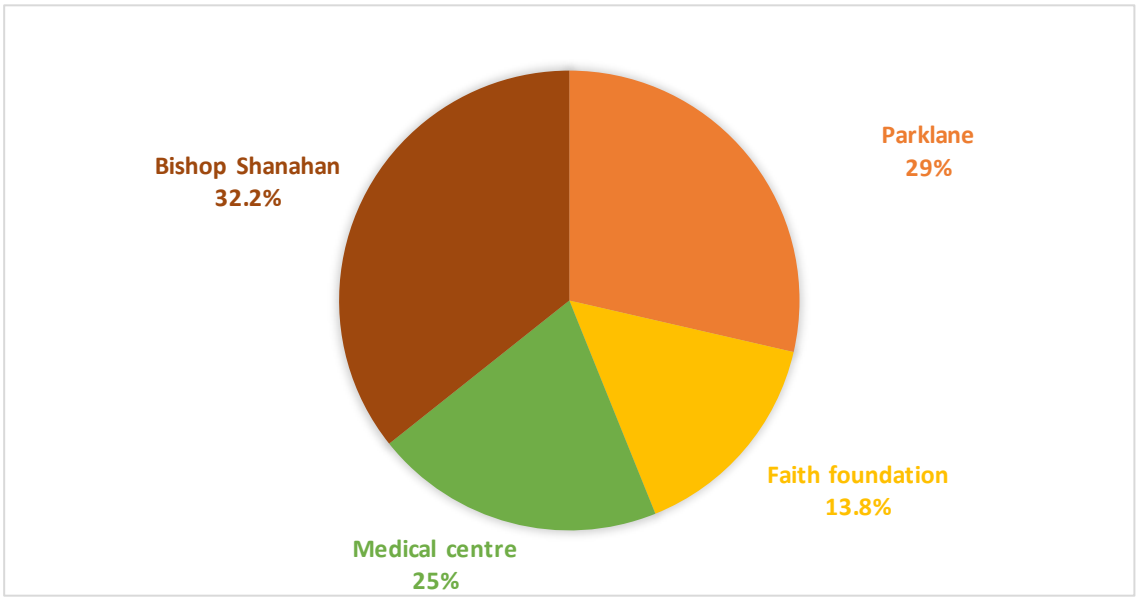

Figure 1: Percentage distribution of isolates

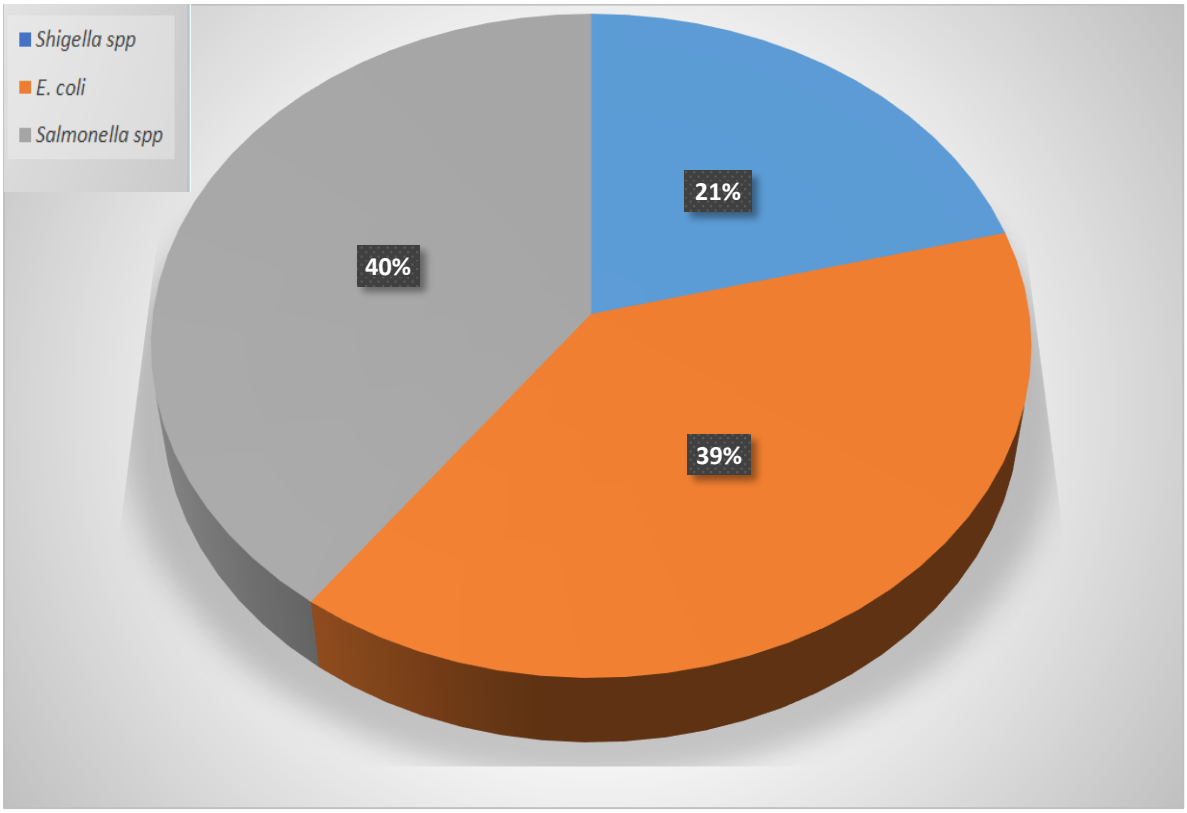

Figure 2: Percentage distribution of the three bacteria isolates

Table 1: Biochemical characteristics of *E. coli* cultured from the pediatric ward of 4 hospitals

Sample number	SSA	Mac-Conkey	EMB	I	MR	VP	C	U
FH1	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
FH8	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
P7	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
P8	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MH1	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MH7	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MH9	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MHII	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MH12	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MH14	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MH15	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MH17	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MH18	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MHS19	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MHS9	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MHS13	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MHS14	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MHS15	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MHS47	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MHS50	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MHS51	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MHS63	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MHS69	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-
MHS78	Slight growth, pinkish	Non-mucoid, dark pink	Greenish metallic sheen with a dark centre	+	+	-	-	-

Key: + = Positive, - = Negative, I = Indole, C = Citrate, U = Urease

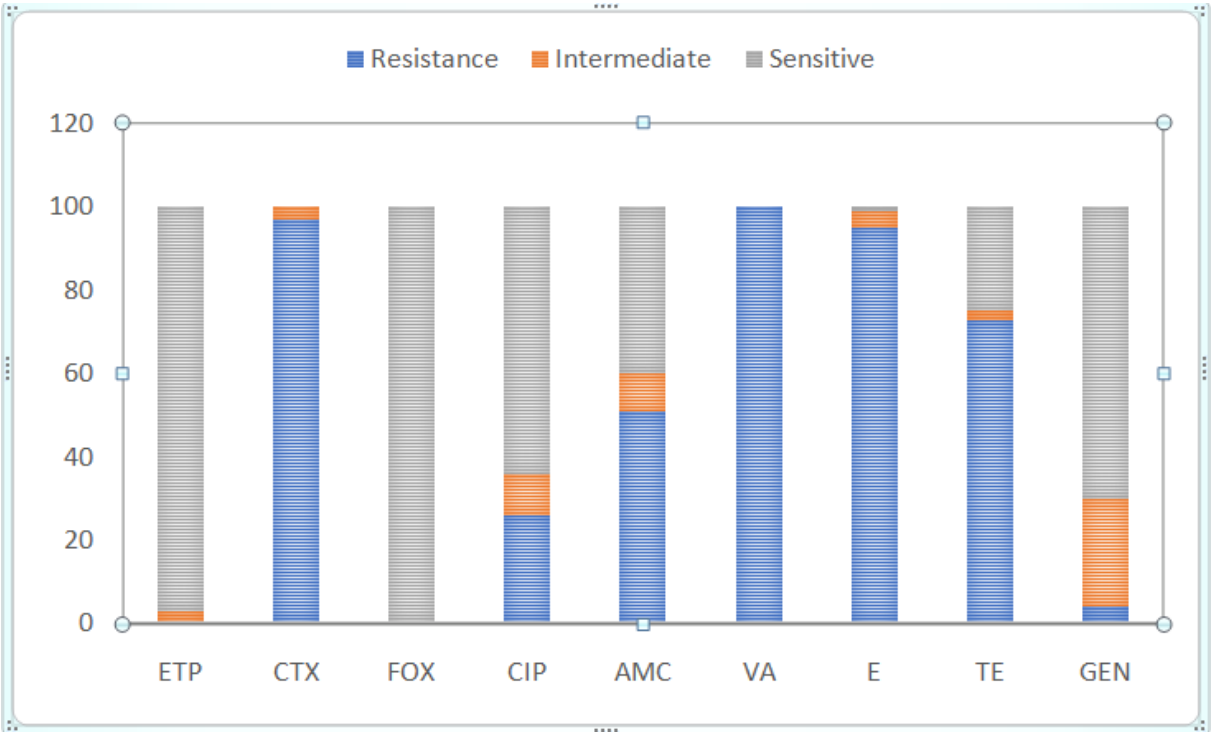

Figure 3: *Percentage distribution of antibiotic susceptibility of E. coli*
Key: ETP = Ertapenem; CTX = Ceftriaxone; FOX = Cefoxitin; CIP = Ciprofloxacin; AMC = Amoxicillin/clavulanic acid; VA = Vancomycin; E = Erythromycin; TE = Tetracycline, GEN =Gentamicin

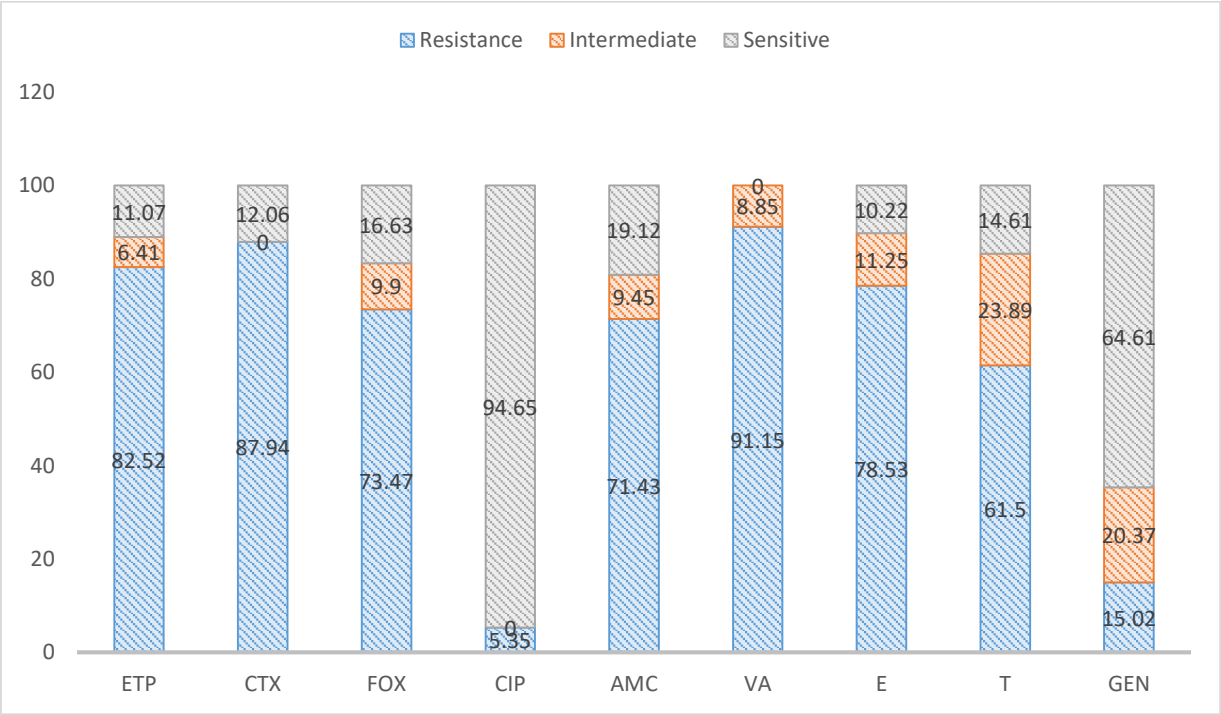

Figure 4: Percentage Distribution of Antibiotics Susceptibility of *Shigella* spp
Key: ETP = Ertapenem; CTX = Ceftriaxone; FOX = Cefoxitin; CIP = Ciprofloxacin; AMC =Amoxicillin/clavulanic acid; VA = Vancomycin; E = Erythromycin; TE = Tetracycline, GEN = Gentamicin

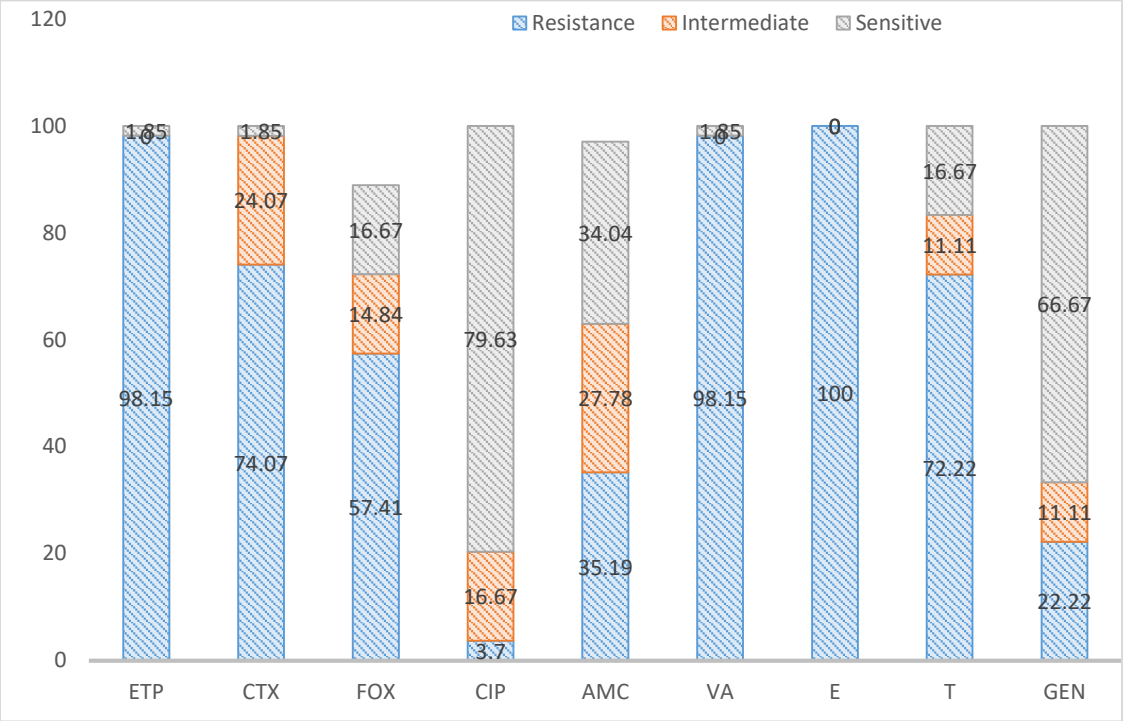

Figure 5: Antibiotic susceptibility pattern of *Salmonella* spp. to different antimicrobial agents
ETP = Ertapenem; CTX = Ceftriaxone; FOX = Cefoxitin; CIP = Ciprofloxacin; AMC = Amoxicillin/clavulanic acid; VA = Vancomycin; E = Erythromycin; TE = Tetracycline, GEN = Gentamicin.

such as viruses, fungi, or protozoa, are much less common [27]. However, among the pathogenic bacteria species, *E. coli, Salmonella*, and *Shigella* are the most common [28]. Therefore, the presence of these organisms confirmed their roles in diarrhoea among children in these clinics.

The isolation of multidrug-resistant *E. coli*, *Shigella* spp, and *Salmonella* spp from diarrhoea samples in this study is similar to the result of other studies [29, 30]. From the samples, *E. coli* showed high resistance to erythromycin and tetracycline. This study's results align with another study conducted in South Africa [31]. The *E coli* isolates also exhibited high resistance to cefotaxime, in contrast to the study carried out in Pakistan that showed that cefotaxime was 100% effective against the organism [32]. This study also is in agreement with the antibiotic susceptibility patterns carried out at Kaduna, which shows that the isolates were resistant to cefotaxime, tetracycline, and amoxicillin [33]. However, the *E. coli* isolates were sensitive to ciprofloxacin, gentamycin, and ertapenem (carbapenem). Carbapenems (ertapenem) are still the first drug of choice in treating severe infections caused by *E. coli*. It has been reported that E. *coli* is still susceptible to carbapenems; hence carbapenems remain a credible option for the treatment of serious infections caused by *E. coli* [34, 35, 36].

Shigella spp exhibited high resistance to cefotaxime, which disagrees with a study in Iran. Their study showed that cefotaxime was effective in treating shigella infection [37], but did not inhibit the growth of *Shigella* spp in the isolates we investigated in the study. In a similar study of antibiotic susceptibility patterns of *Shigell*a spp in Oyo State in Nigeria, it was also observed that *Shigella* spp were resistant to erythromycin, vancomycin and sensitive to ciprofloxacin, gentamycin, and ceftriaxone [38].

All tested isolates were resistant to erythromycin. This study also has proven that ciprofloxacin is still a potent drug of choice in the incidence of salmonella outbreaks. More so, erythromycin, ertapenem, and vancomycin showed high ineffectiveness and should not be recommended as a treatment option in an episode of salmonella infection in the study location. The resistance of some of these organisms to ertapenem (carbapenem) is a serious cause for worry, given that carbapenem are used as a last resort in serious bacterial infections that do not respond to other common antibacterial agents. Though ciprofloxacin was a better option for the study, the age of the patient should be put into consideration when administering this drug to avoid adverse effects.

CONCLUSION

The misuse and abuse of antibiotics, especially in rural areas of our communities in developing countries, should be discouraged. This is because the practice has been linked to increasing antimicrobial resistance by pathogens. There is also a need for increased awareness of the importance of personal hygiene, and nursing mothers should be cautious of what they feed their children to avoid diarrhoea. The immediate use of health facilities with trained personnel is the sure way to reduce the morbidity and mortality associated with childhood conditions like diarrhoea. Robust laboratory investigations like antibiotic sensitivity testing may guide the clinician on the best

therapeutic option, as seen in the results of our study. The study showed that ciprofloxacin and gentamicin are the most effective drugs for treating diarrhoea caused by *E.coli*, Shigella spp, and Salmonella spp in the study communities in Enugu State, Nigeria. However, ertapenem is also very effective in managing *E. coli* implicated in diarrhoea in the study.

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AUTHORS' CONTRIBUTION

E.S.A. conceptualized and drafted the guideline. E.C.O. did the statistical analysis and initial draft of the manuscript. BDP was involved in sample collection and microbiological studies. ECE was involved in sample collection and microbiological culture preparations. AIS did sample collection and data analysis. OCA was involved in manuscript editing and reviews, and AAA designed and supervised the research.

CONFLICT OF INTEREST

The authors wish to state that there was no conflict of interest in carrying out the research.

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